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(54) Title: NOVEL ANTI-INFLAMMATORY PEPTIDES

(57) Abstract: The present invention provides novel anti-inflammatory peptides having the amino acid of the SEQ ID NO:1(KVLDPVKG); SEQ ID NO:2(KVLDGQDP) or SEQ ID No:3(DPVKG) containing inhibitory effects of transglutaminase and phospholipase A_2, their derivatives, pharmaceutical compositions containing the aforesaid peptides, and methods for preventing inflammation by using the same. The peptides, derivatives thereof, analogs thereof and the pharmaceutical compositions containing the same of the present invention show the higher anti-inflammatory activities than the existing known peptides or steroidal anti-inflammatory drugs, such as dexamethasone.

NOVEL ANTI-INFLAMMATORY PEPTIDES

TECHNICAL FIELD

The present invention provides novel anti-inflammatory peptides containing inhibitory effects of transglutaminase and phospholipase A_2, their derivatives, pharmaceutical compositions containing the aforesaid peptides, and methods for preventing inflammation by using the same.

BACKGROUND OF THE INVENTION

An inflammatory reaction is a complex biochemical and cytological phenomena that are manifested physiologically in tissue by edema, pain and leukocyte infiltration. The most effective drugs for the inflammation are glucocorticoids. Glucocorticoids, conventional anti-inflammatory steroidal drugs, have been proved to exhibit an excellent activity against rheumatoid arthritis and others by inhibiting or preventing various inflammatory reactions which is caused by radioactive, mechanical, chemical, infectious and immunological stimulation.

However, as the steroidal anti-inflammatory drugs are used widely, recently various harmful side effects caused by their abuse have resulted in serious problems. The steroidal anti-inflammatory drugs clinically causes two categories of side effects: the symptoms caused by a sudden break after a long-term administration and those others caused by too much use for a long time. After long-term administration of adrenocortical hormones, abruptly stopping it causes symptoms such as general prostration, fever, myalgia, arthralgia, and decrease of appetite, etc. as an outcome of the renal paresis. It also causes the increased opportunity for bacterial and viral infections, body weight increase, body form change and insomnia. So, there have been several problems for their applications, and thus it is desirable to develop a new anti-inflammatory drug without causing any side effects. To avoid these side effects, we need to develop specific inhibitors at certain inflammatory pathway. PLA_2 is the initial step enzyme to generate arachidonic acid from phospholipids that causes that inflammation at several steps later as prostaglandins and leukotrienes. Therefore, blocking of PLA_2 activation may be the best way to prevent inflammation. Indeed

steroid is a powerful therapeutic approach although precise mechanism is not yet clear. One of the key mechanisms of steroid has been proposed that anti-inflammatory effect is mediated by induction of anti-inflammatory proteins. Glucocorticoid induces many proteins such as lipocortins, inhibitory proteins of phospholipase A_2 (PLA_2)(Flower, R. J. et al., *Nature* 278, 456-459, 1979). Numerous studies have revealed that their anti-inflammatory effects are mediated by the induction of lipocortins (Flower, R. J., et al., *Adv. Inflamm. Res.* 7, 61-69, 1984) and uteroglobins (Miele et al., *Endocr. Rev.* 8, 474~490, 1987). Lipocortins (annexins) are a class of proteins that share structural and functional features. In the functional feature, Miele et al. identified a region of sequence similarity between uteroglobin and lipocortin-1. Further they designed several synthetic peptides corresponding to the region of highest similarity between uteroglobin and lipocortin-1: nonapeptides, so called antiflammins (AFs), corresponding to uteroglobin residues 39-47 and lipocortin-1 residues 246-254. Both peptides were shown to be phospholipase A_2 (PLA_2) inhibitors in vitro and were effective in a classic model of acute inflammation in carrageenan-induced rat footpad edema (Miele et al., *Nature* 335, 726-730, 1988). However, it is controversial whether or not AFs have any inhibitory effect on PLA_2 as well as anti-inflammatory activity in vivo (Hope, W.C., et al. *Agents Actions* 34, 77-80, 1991; Marki, F., et al. *FEBS Lett.* 264, 171-175, 1990; Van Binsbergen, J., et al *FEBS Lett.* 247, 293-297, 1989). The existing antiflammin or PLA_2 inhibitor alone was not able to show potent anti-inflammatory effects like dexamethasone.

In the structural feature of lipocortins, most of them behave as extrinsic membrane proteins, which bind reversibly to phopholipid membranes in a manner that depends on calcium ions. Also during the epithelial cell differentiation, lipocortin-1 (annexin-1) becomes incorporated into the cornified cell envelope via cross-linking by transglutaminase (TGase) and cannot be extracted by SDS (Moore, K. G., et al *Exp. Cell Res.* 200, 186-195, 1992; Lee, C. H., et al., *FEBS Lett.* 477, 268-272, 2000).
In the oral epithelium, it was found by sequencing proteins that lipocortin-1 constitutes about 10% of all the barrier envelope proteins. Thus lipocortin-1 itself possibly involves in the barrier formation as a major component containing anti-inflammatory function.

Another way of blocking PLA_2 can be inhibition of PLA_2 stimulation. Interestingly there is a report that PLA_2 was stimulated by TGase (Cordella-Miele, E, Miele, L, & Mukherjee, A. B. *J. Biol. Chem.* 265, 17180-17188, 1990). The increase of catalytic activity of PLA_2 was due to conformational change with intra molecular cross-linking

by TGase. We have discovered that the inflammatory cytokines such as IFN- or TNF- could increase the expression of TGase (Kim, S.-Y., Jeong, E.-J., & Steinert, P.M. *J. Interferon and Cytokine Res.* in press, 2002). Consequently increase of TGase may cause increase of PLA_2 activity. Therefore, TGase inhibitors could inhibit inflammation through inhibition of PLA_2 stimulation.

As a result of the careful researches by the present inventors, sequence similarity between AFs and TGase substrate domain of elafin, which represents a core tetra peptide KVLD in AFs and DPVK in elafin. Elafin, also known as SKALP (skin-derived antileukoproteinase), is 6 kDa preproform that can be activated to 3 kDa active form by proteolysis, which is specific and potent inhibitor of polymorphonuclear (PMN) cell-derived serine proteinases such as elastase and proteinase-3 (Molhuizen, H. O. F. et al., *J. Biol. Chem.* 268, 12028-32, 1993). Although KVLD is inactive as a PLA_2 inhibitor (Miele et al., *Nature* 335, 726-730, 1988), which could be active for the TGase inhibitor. The present inventors have also discovered that when an antiflammin contains lysine residues as an acyl acceptor, it competes with TGase substrate, and succeeded in synthesizing novel peptides from antiflammin based on this finding.

SUMMARY OF THE INVENTION

The objective of the present invention is to provide novel peptides having an excellent anti-inflammatory activity in comparison with the existing antiflammin, and without causing any of the side effects inherent in the steroidal anti-inflammatory drugs and also their medical use. In other words, the present invention provides synthetic peptides having amino acid sequence, SEQ ID NO: 1, 2 or 3 of the sequence listing.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effects of the peptides of the present invention inhibiting the activity of PLA_2 in vitro;

Fig. 2 is a graph showing the effects of the peptides of the present invention inhibiting the activity of TGase in vitro;

Fig. 3 is a graph showing the effects of the peptides of the present invention inhibiting the activity of PLA_2 activated by TGase in vitro;

Figs. 4 and 5 are graphs showing the results of the experiments with the peptides of the present invention for anti-inflammatory activity in the allergic conjunctivitis model using guinea pigs; and

Fig. 6 is showing H & E staining of conjunctiva. Eosinophils in the standardized three fields of conjunctival epithelium and the stroma were counted (X400).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides peptides and peptide derivatives having the amino acid sequence of the SEQ ID NO:1 (KVLDPVKG); SEQ ID NO:2 (KVLDGQDP) or SEQ ID No:3 (DPVKG) of the sequence listing, and analogous peptides, which exhibit anti-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

The present invention also provides pharmaceutical compositions comprising said peptides, peptides derivatives and peptides analog and a method for inhibiting inflammation utilizing the same.

The peptides of the present invention can vary within the range wherein their activity is maintained. At least one side chain amine group of the peptides for example, can be acylated or arylated, or at least one hydroxyl group of said peptides can be esterified to an alkyl group or an aryl group.

In one embodiments, the present invention provides for analog and/or derivatives of said peptides(SEQ ID NO:1, 2 or 3) as anti-inflammatory agents, such as those in which the amino-terminal end of the peptide is modified by addition of an R-(C=O)- group in which R is selected from the group consisting of an alkyl, a cycloalkyl, an aryl and heteroaryl, wherein the aryl or heteroaryl is either unsubstituted or substituted with a halogen, methoxy, amino or alkyl functional group; or in which the carboxy-terminal end of the peptides can also be modified by addition of an R'-group in which R'-is selected from the group consisting of an amine, an amide, an alkyl ester, a cycloalkyl ester, an aryl ester, or a heteroaryl ester, wherein the aryl ester or heteroaryl ester can be either unsubstituted or substituted with a halogen, methoxy, amino, or alkyl functional

group, or in which both ends of the peptide can be so modified, provided that said analogs and/or derivatives exhibit anti-inflammatory activity. It is well known to those who skilled in the art that these analogs or derivatives are also within the scope of the present invention. As used herein, the terms "aryl" and "aryl ester" are intended to encompass groups containing a 6- or 7-unit ring structure, and include, e.g., pyridinium, imidazolium and quinoxaline groups.

In other embodiments, the present invention provides for analogs of said peptides (SEQ ID NO:1, 2 or 3) as anti-inflammatory agents in which any one or more of the amino acid residues of said peptides(SEQ ID NO:1, 2 or 3) can be substituted by different amino acid analogs or mimic, e.g., to produce carbazates or tertiary centers, the incorporation of which serves to avoid or reduce proteolytic cleavage of the peptide, provided that said analogs exhibit anti-inflammatory activity.

The peptides of the present invention may be prepared by any method known in the art. For example, and not by way of limitation, the peptides may be synthesized: (i) by cleavage from a larger peptide; (ii) by recombinant DNA expression methods; and (iii) by chemical synthesis, including solid phase techniques as described by Barany and Merrifield(1980, in "*The Peptides*" Vol 2. Gross and Meienhofer. Eds., Academic Press, N.Y.), preferably by an automatic peptide synthesizer.

As to the recombinant DNA expression method, the conventional method comprising the steps of synthesizing the DNA corresponding to the amino acid sequence of the peptides of the present invention; attaching a linker having restriction enzyme cleavage site to the termini of said DNA; and then preparing a recombinant vector by ligating said DNA-linker molecule into a conventional vector having a regulatory promoter.

The peptides of the present invention can be produced, by transforming the resultant recombinant vector into a suitable host and then expressing the peptides within the transformed organism by a biotechnological process. The examples of the vectors include a conventional vectors such as made from plasmid, cosmid, YAC, virus etc. and the examples of the hosts include bacteria, yeast, fungi, animal cells, plant cells, and others. For the purposes of the separation and purification of the resultant peptides, signal sequences for extracellular destination can be ligated to the synthetic DNA, or the peptides can be produced in the form of fusion proteins by linking a portion of a gene or

whole gene which is originated from the host to the aforementioned DNA. After separation and purification, the fused proteins can be cleaved and separated by chemical or enzymatical procedures.

The peptides of the present invention exhibit potent anti-inflammatory activity, and may also be administered into a subject together with biologically active agents, such as biologically active compounds or other peptides, or in parallel with other kinds of anti-inflammatory drugs.

Further, the present invention provides a pharmaceutical composition for anti-inflammation, comprising an effective amount of the peptide, peptide derivative or peptide analog of the aforementioned peptides and pharmaceutically acceptable carrier. The said pharmaceutical compositions of the present invention may contain one or more of the conventional pharmaceutically acceptable solvents, surfactants, oil or anti-oxidizers for their chemical stability. The said compositions contain the said synthetic peptides by 0.00001~50% in the basis of weight of the total composition, and preferably by 0.001~30%. If it is less than 0.00001 wt %, the desirable activity of the present invention is insignificantly low, if more than 50 wt%, the desirable activity of the present invention doesn't increase correspondingly to the increase of the amount.

The compositions of the present invention may be administered by any suitable and accepted route of drug administration, including intravenous, subcutaneous, intradermal, intranasal, inhalation (e.q., by lung aerosol or lavage), intramuscular, intraocular, intraperitoneal injection, peritoneal lavage, cardiac puncture, cardiac catheter injection, oral, intrathecal or intraventricular injection, spinal column or cranial cavity injection, vaginal or rectal(e.g., by suppository), dermal patch or topical ointment, and may be comprised in any suitable pharmaceutical carrier, including aquous solution, microcapsules, liposomes, or via a sustained-released implant, including hydrophilic or hydrophobic carrier-based implants.

The administration dosage will differ depending on age, gender, body weight, symptoms, treatment effect, administration route, treatment time and substance administrated with. But it is desirable to administer in an effective amount to inhibit the inflammation, desirably 0.001 g ~ 2 g/kg for each time for adults. It is well known to the art that the peptides may be modified by aforementioned method to prevent proteolysis during their delivery, depending on the administration route, for example

oral administration.

Such a composition may also include adjuvants, such as preservatives, humectants, emulsifiers, dispersers and stabilizers (for example, arginine and aspartic acid).

The present invention also provides a method for inhibiting an inflammatory response that is associated or caused by inflammatory diseases, comprising the step of exposing humans or animals to an effective amount of the peptide, peptide derivative or peptide analog aforementioned.

The examples of the inflammatory response that is associated or caused by inflammatory diseases including autoimmune diseases such as ulcerative colitis, rheumatoid arthritis, scleroderma, inflammatory lung disease, celiac disease, systemic lupus, myasthenia gravis and diabetes; skin allergy, pimples or trauma. The method of the present invention may be also used in all of the diseases or symptoms in which the peptide of the present invention are effective, such as various degenerative diseases, painful diseases or nervous diseases.

The invention will now be explained in greater detail by way of the following examples, with understanding that the invention is in no way restricted by these examples.

EXAMPLES

Example 1

(1) Synthesis of the peptides

According to the sequences described in Table 1, the peptides of the present invention were synthesized by an automatic peptide synthesizer, and the synthesized peptides were purified by using a C8 reverse phase high performance liquid chromatography.

Further, by using abovementioned method, known peptides such as antiflammin were synthesized, and their sequences are as shown in Table 2.

Table 1.

Peptide	Amino acid sequence
SEQ ID NO:1	KVLDPVKG
SEQ ID NO:2	KVLDGQDP
SEQ ID NO:3	DPVKG

Table 2.

Peptide	Amino acid sequence
SEQ ID NO:4(PLA_2 inhibitor)	PKLMEI
SEQ ID NO:5(PLA_2 inhibitor)	GTLAKKLT
SEQ ID NO:6(antiflammin)	SHLRKVFDK
SEQ ID NO:7(antiflammin)	HDMNKVLDL
SEQ ID NO:8(antiflammin)	MQMKKVLDS
SEQ ID NO:9(antiflammin)	KVLD
SEQ ID NO:10(TGase inhibitor)	PVKG
SEQ ID NO:11(TGase inhibitor)	GQDP

(2) Inhibitory effect of synthetic peptides on PLA_2 and TGase activities in vitro

Inhibitory effect of synthetic peptides on PLA_2

It has been examined whether synthetic peptides contain inhibitory effect on PLA_2 activity. A established PLA_2 assay method was used to determine the enzymatic activity by measuring the release of [^{14}C]-arachidonic acid from 1-acyl-2-[1-^{14}C]arachidonic acid-glycerophospho-ethanolamine (Biochem. Pharmacol. 54, 259-268, 1997).

To determine the inhibitory activity, 0.18 Unit PLA_2 (Bovine pancreas, EC 3.1.1.4, Sigma; 1.0 unit will hydrolyze 1.0 mole of L- -phosphatidylcholine to L- -lysophosphatidylcholine and a fatty acid per min. at pH8.0 at 37 °C) was preincubated for 15 minutes with the synthetic peptides (1 x 10^{-8} M) at 37 °C in 60 l before adding 1-acyl-2-[1-^{14}C]arachidonic acid-glycerophospho-ethanolamine. The control was also PLA_2 preincubated with buffer. After pre-incubation, PLA_2 activity was assayed using sonicated liposomes, prepared as established method (Biochem. Biophys. Acta. 1083, 80-88, 1991). 40 l of 1-acyl-2-[1-^{14}C]arachidonic acid-glycerophospho-ethanolamine (about 2 x 10^5dpm) was added in pre-incubated mixture, and incubated for 1 hr at 37°C.

The reaction was terminated by adding 0.75 ml of Dole's reagent (78% n-heptane, 20% propan-2-ol, and 2% 2M aqueous H_2SO_4), and the liberated [^{14}C]-arachidonic acid was extracted as follows. 0.25 ml of H_2O was added into the incubated mixture, vortexed, and centrifuged at 1,200 x g for 5 min. Then upper phase was transferred to a new tube that contained 100 mg of silica gel (230-400 mesh, Sigma) and 0.75ml of n-haptene. The sample was vortexed and centrifuged at 1,200 x g for 5 min. The supernatant was collected for scintillation counting (Biochem. Pharmacol. 54, 259-268, 1997). Values are means for three determinations (SD<10%).

For inhibitory test of the peptides given, each value is the mean of data from three separate experiments (S.D. < 10%). The value of PLA_2 activity incubated without peptides was determined as 100% PLA_2 activity that is 5.57 pmole arachidonyl/hr. % Inhibition was shown in Fig.1.

Inhibitory effect of synthetic peptides on TGase

It is needed to inhibit the activity of TGase in order to secure maximum anti-inflammatory activity since the TGase activates PLA_2. A established TGase assay method was used to determine the enzymatic activity by measuring the incorporation of [1,4]^{14}C-putrescine into succinylated casein (Folk JE., et al., *Transglutaminase. Methods in Enzymol* 1985; 113; 358-375).

To determine the inhibitory activity, 0.001 U TGase (Guinea pig liver, EC 2.3.2.13, Sigma; 1.0 unit will catalyze the formation of 1.0 mole of hydroxamate per min. from N- -CBZ-Gln-Gly and hydroxylamine at pH 6.0 at 37 °C) was preincubated for 15 minutes with the synthetic peptides in 0.1 ml (1 x 10^{-8} M) at 37 °C before adding the TGase /peptide mixture to the substrate solution (0.5 ml) containing 0.1M Tris-acetate pH 7.5, 1% succinylated casein, 1 mM EDTA, 10 mM $CaCl_2$, 0.5% lubrol PX, 5mM DTT, 0.15M NaCl and 0.5 mCi ^{14}C-putrescine [Dupont-New England Nuclear, Wilmington, Germany (118 Ci/mole)]. Following incubation at 37 °C for one hour, the reaction was terminated by addition of 4.5 ml of cold (4 °C) 7.5% TCA. The TCA-insoluble precipitates were collected onto GF/A glass fiber filters, washed with cold 5% TCA, dried and counted. The resulting TGase inhibiting activity was as shown in Fig 2. The control was also TGase preincubated with buffer. Values are means for three determinations.

For inhibitory test of the peptides given, each value is the mean of data from three separate experiments (S.D. < 10%). The value of TGase activity incubated without peptides was determined as 100% TGase activity that is 4.89 pmole putrescine/hr. % Inhibition was shown in Fig.2.

Inhibitory effect of synthetic peptides on PLA_2 activated by TGase

A series of synthetic peptides (1×10^{-8} M) were pre-incubated with 0.001 Unit TGase (Guinea pig liver, Sigma) in 60 l of assay buffer (75mM Tris-Cl, pH 9.0, 5mM $CaCl_2$, 1mg/ml fatty acid free bovine serum albumin) for 15 min at 37°C. We employed a negative control as assay buffer without TGase, and a positive control as TGase without peptides. After pre-incubation, 40 l of 1-acyl-2-[1-^{14}C] arachidonic acid-glycerophospho –ethanolamine (about 2×10^5dpm) was added in pre-incubated mixture, and incubated for 1 hr at 37°C. The reaction was terminated by adding 0.75 ml of Dole's reagent, and followed the same procedure described as above.

PLA_2 activity was increased about 2-fold after TGase 2 treatment, demonstrating that TGase activates the PLA_2 activity. For inhibitory test of the peptides given, each value is the mean of data from three separate experiments (S.D. < 10%). The increased PLA_2 activity by TGase was determined as 100% that was 11.22 pmole arachidonyl/hr. % Inhibition was shown in Fig.3.

As shown in Fig. 1, 2 and 3, the R1 and R2 peptides of the present invention significantly reduced the PLA_2 and TGase activities in comparison with the peptides known in PLA_2 inhibitors.

Example2: In Vivo measurement of anti-inflammatory activity.

The peptides of the present invention were analyzed in experimental model of allergic conjunctivitis to ragweed (giant ragweed; *Ambrosia trifida*) in guinea pig. Hartley strain of guinea pig was selected according to previous reports of conjunctival anaphylaxis (Calonge M., et al., *Acta Ophthalmol* 68; 470-476, 1990) and females were chosen based on the sex dimorphism of ocular mucosal immunity with the protective role of male hormones (Saruya S., et al., *Act Soc Ophthalmol Jap* 72; 833-845, 1968).

The conjunctival redness and edema are main signs of seasonal allergic conjunctivitis and eosionphil infiltration is the hallmark of the conjunctival histology in patients with allergic conjunctivitis (Abelson MB., et al., *Arch Ophthalmol.* 101; 555-556, 1983; Butrus SI., et al., *Int. Ophthalmol. Clin.* 28; 324-328, 1988)

(1) Animals

86 Hartley female guinea pigs, 200-250g were housed in the animal facilities of the Asan Institute for Life Sciences (Korea), given guinea pig chow and water ad libitum, and cared for in accordance with the Declaration of Helsinki and the National Institute of Health Guide to the Care and Use of the Laboratory Animals (Korea).

(2) Immunization and challenge

Giant ragweed pollen, *Ambrosia trifida*, 1.0-1.5 mg (Sigma) was delivered into the nostrils and the inferior conjunctival fornices of the 84 animals on days 1 to 5 and 8 to 12, once a day with an Eppendorf micropipette calibrated to 10μl. On day 15, immunized guinea pigs were divided 14 groups, 6 for each group. 11 anti-inflammatory peptides including new recombinants, dexamethasone eye drop (Maxidex, 0.1% dexamethasone, Alcon) and anti-histamine eye drop (Livostin, levocabastine, Janssen) were used for 13 groups, and no treatment for one group. Normal controls were employed as 6 non-immunized and non-treated guinea pigs. All immunized guinea pigs were challenged with 1.0-1.5mg of ragweed pollen powder delivered to the inferior conjunctival fornice on day 15. All the procedures were followed experimental model of allergic conjunctivitis described by Merayo-Lloves et al (*Experimental model of allergic conjunctivitis to ragweed in guinea pig. Curr. Eye Res.* 14; 487-494, 1995).

(3) Treatment using peptides, dexamethasone eye drop and anti-histamine eye drop

Anti-inflammatory peptides were resolved in sterile saline (100μmol/40μl concentration). All peptides and dexamethasone eye solutions were challenged 3 hour prior to the last pollen application on day 15, 10 minutes after pollen application, and 4 times thereafter at the 3 hour interval.

(4) Clinical evaluation

Conjunctival edema and redness were judged in room light and under an operating

microscope (Zeiss, Germany). The evaluation was performed in a masked fashion at 20 min after allergen challenge and each clinical sign was scored on a scale 0-4+ (0 absent, 1+ minimal, 2+ mild, 3+ moderate, 4+ severe), adapted for guinea pig from previous report (Merayo-Lloves et al: *Experimental model of allergic conjunctivitis to ragweed in guinea pig. Curr. Eye Res.* 14; 487-494, 1995). Clinical scores of each group (12 eyes in each group) were analyzed.

(5) Histology

The orbits were excentrated after the animals were killed with CO_2 asphyxia, and specimens were prepared for light microscopic examination. Sectioned specimens were stained with hematoxylin-eosin.

Eosinophils in the standardized fields of conjunctival epithelium, the immediate subepithelial, and the stroma were counted in three non-consecutive fields (magnification x400) for each treatment in each experiment (12 eyes in each group).

(6) Results

Clinical signs

Conjunctival redness and edema scores are shown in Fig. 4 and Table 3.

Clinically, RKLMEI (SEQ ID NO:4), SHLRKVFDK (SEQ ID NO:6), MQMKKVLDS (SEQ ID NO:8), KVLDGQDP (SEQ ID NO:2), PVKG (SEQ ID NO:10), DPVKG (SEQ ID NO:3), and dexamethasone (a steroidal anti-inflammatory drug) showed statistically significant difference from other groups (n=12 , $P<0.01$, SD < 10%, Mann-Whitney test). Especially, the one of the peptide of the present invention (SEQ ID NO:2), dexamethasone and Livostin (an anti-histamine eye drop) were most effective.

Table 3. Statistical evaluation of clinical scores (Mann-Whitney test)

Name	Sequence	Compared to No treatment group	to Dexamethasone	to Livostine
P1	RKLMEI	0.009	0.001	0.003
P2	GTLAKKLT	0.279	0.000	0.000
A1	SHLRKVFDK	0.003	0.007	0.009
A2	HDMNKVLDL	0.382	0.002	0.003
A3	MQMKKVLDS	0.000	0.089	0.043
A4	KVLD	0.082	0.000	0.000
R1	KVLDPVKG	0.965	0.000	0.000
R2	KVLDGQDP	0.000	0.796	0.515
E1	PVKG	0.001	0.017	0.012
E2	DPVKG	0.000	0.050	0.039
E3	GQDP	0.016	0.000	0.002
Dexamethasone		0.000		

Histological signs

The histological results are also shown in Fig. 5, Fig. 6 and Table 4. Most anti-inflammatory peptides except HDMNKVLDL (SEQ ID NO:7) inhibited eosinophil infiltration (n=12, P<0.01, SD<10%, Mann-Whitney test). Especially, KVLDGQDP (SEQ ID NO:2) and DPVKG (SEQ ID NO:3) of the present invention, dexamethasone (a steroidal anti-inflammatory drug) and Livostin (an anti-histamine eye drop) showed statistically significant difference from other groups. Among those, KVLDGQDP (SEQ ID NO:2) of the present invention was the most effective.

Table 4. Statistical evaluation of eosinophil count (Mann-Whitney test)

Name	Sequence	Compared to No treatment group	to Dexamethasone	to Livostine
P1	RKLMEI	0.002	0.000	0.000
P2	GTLAKKLT	0.004	0.000	0.000
A1	SHLRKVFDK	0.000	0.004	0.006
A2	HDMNKVLDL	0.161	0.000	0.000
A3	MQMKKVLDS	0.007	0.000	0.000
A4	KVLD	0.001	0.028	0.045
R1	KVLDPVKG	0.002	0.001	0.002
R2	KVLDGQDP	0.000	0.001*	0.002*
E1	PVKG	0.002	0.000	0.000
E2	DPVKG	0.000	0.028	0.060
E3	GQDP	0.001	0.007	0.000
Dexamethasone		0.000		0.932

* better than dexamethasone and Livostine

As shown herein, the peptides of the present invention (SEQ ID NO:1, 2 or 3) have exhibited a excellent anti-inflammatory activity in comparison with other known peptide sequences. Especially, SEQ ID NO:2 and 3 of the present invention have exhibited much higher activity than the steroidal anti-inflammatory dexamethasone. Thus, the peptides of the present invention (SEQ ID NO:1, 2 or 3) show a excellent anti-inflammatory activity without showing any of the harmful side effects of the steroidal anti-inflammatory drugs, such as dexamethasone.

EFFECT OF THE INVENTION

The peptides of the present invention (SEQ ID NO:1, 2 and 3 of the sequence listing) are novel, show much higher anti-inflammatory activities than the conventional antiflammins and other anti-inflammatory drugs, thus are useful for pharmaceutical purposes.

CLAIMS

What is claimed is

1. A peptide having the amino acid sequence of the SEQ ID NO:1 and having anti-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

2. A peptide having the amino acid sequence of the SEQ ID NO:2 and having anti-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

3. A peptide having the amino acid sequence of the SEQ ID NO:3 and having anti-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

4. A derivative of the peptide of any one of claims 1, 2 and 3, wherein at least one side chain amine group of said peptide is acylated or arylated, or at least one hydroxyl group is esterified to an alkyl group or an aryl group, and which derivative exhibits anit-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

5. An analog of the peptide of any one of claims 1, 2 and 3, wherein said analog contains at least one amino acid mimic, which serves to reduce proteolytic cleavage of the analog as compared to the peptide, and which analog exhibits anti-inflammatory activity and inhibitory effects of transglutaminase and phospholipase A_2.

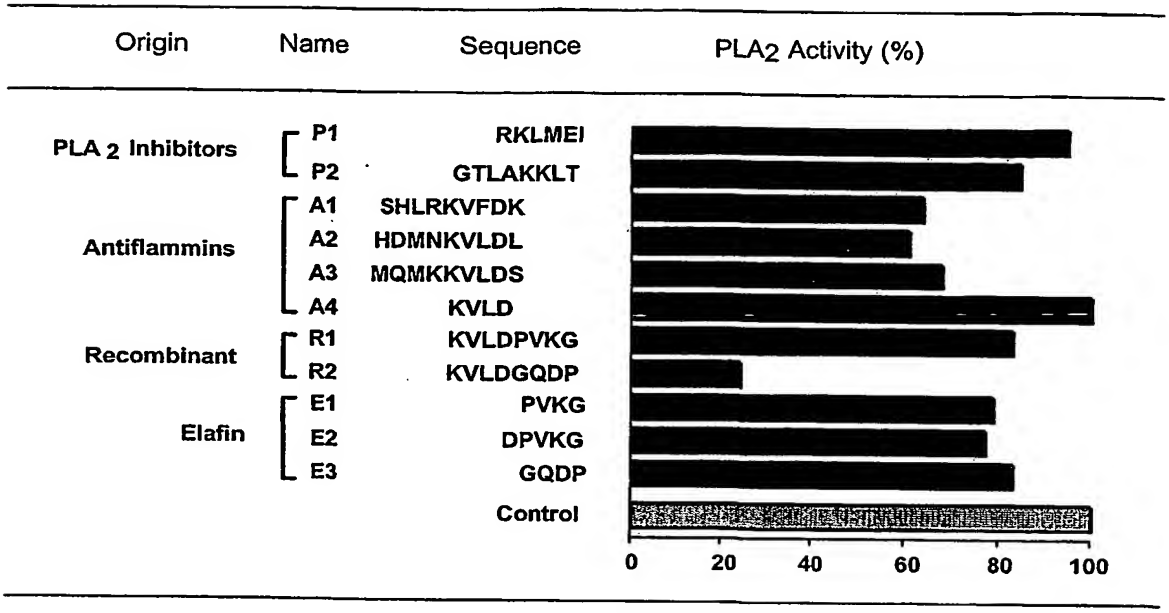
6. A pharmaceutical composition for anti-inflammation and inhibitory effects of transglutaminase and phospholipase A_2, comprising the peptide, peptide derivative or peptide analog of any one of claims 1 to 5 and pharmaceutically acceptable carrier.

7. A method for inhibiting transglutaminase and inflammatory response that is associated or caused by an inflammatory disease, comprising the step of exposing humans or animals to an effective amount of the peptide, peptide derivative or peptide analog of any one of claims 1 to 5.

8. The method of claim 7, wherein said inflammatory disease comprises autoimmune diseases such as ulcerative colitis, rheumatoid arthritis, scleroderma, inflammatory lung disease, celiac disease, systemic lupus, myasthenia gravis and diabetes; various allergic or immune diseases such as skin allergy, pimples or trauma.

9. The method of claim 7, wherein said inflammatory response comprises degenerative diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease; painful diseases or nervous diseases.

Fig. 1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P2
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A2
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A3
MQMKKVLDS
A4
KVLD
Recombinant
R1
KVLDPVKG
R2
KVLDGQDP
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20
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60
80
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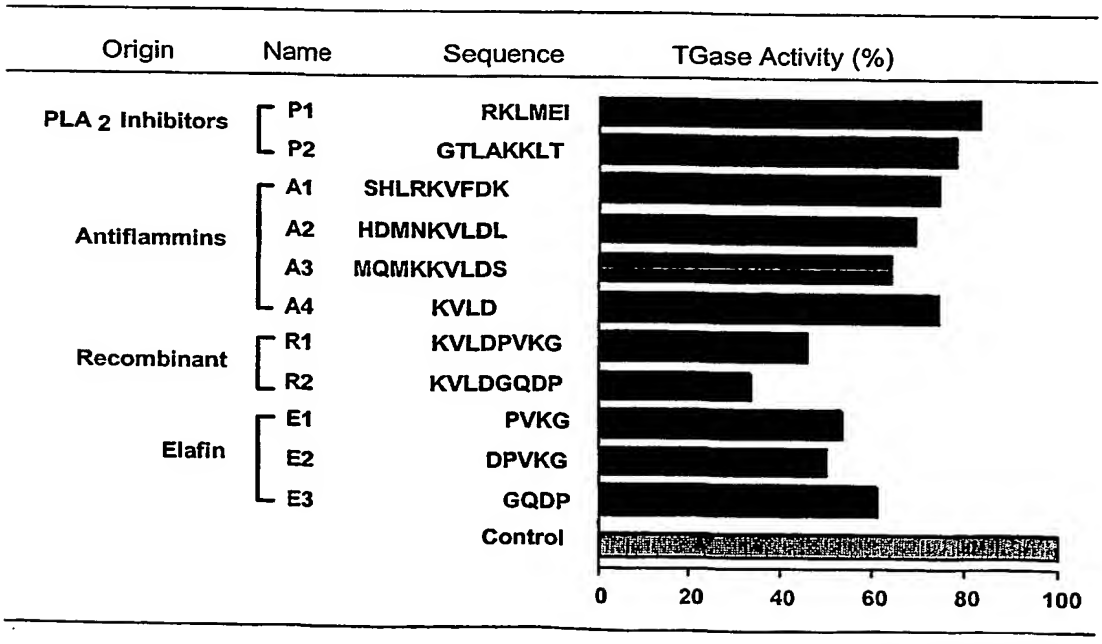
Fig. 2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MQMKKVLDS
A4
KVLD
Recombinant
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R2
KVLDGQDP
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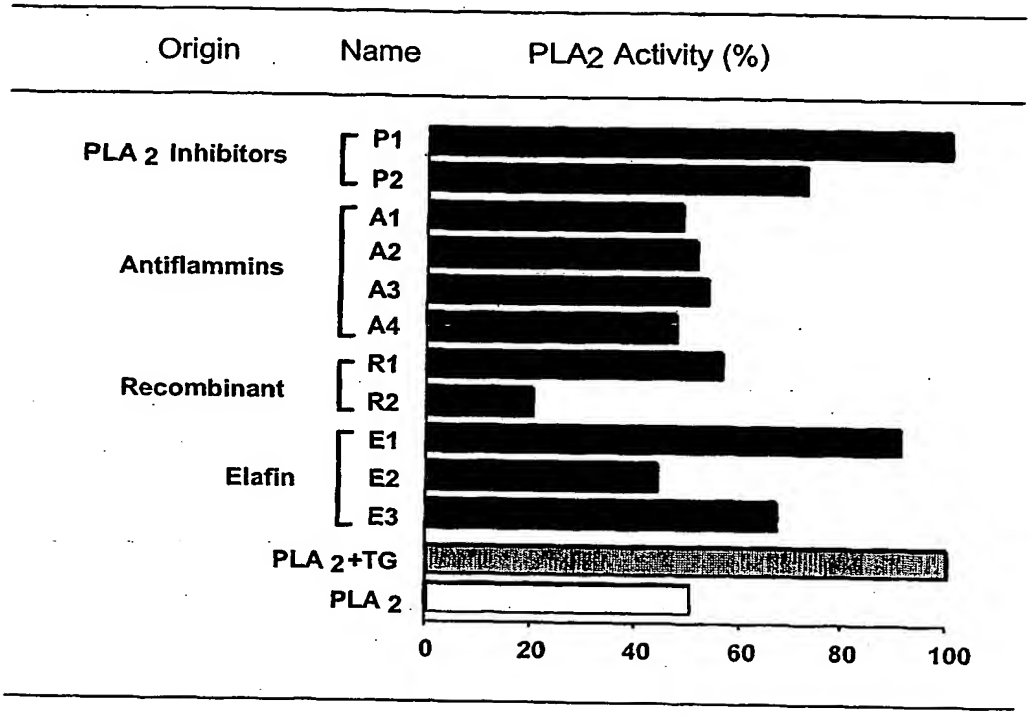
Fig. 3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PLA 2+TG
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Fig. 4

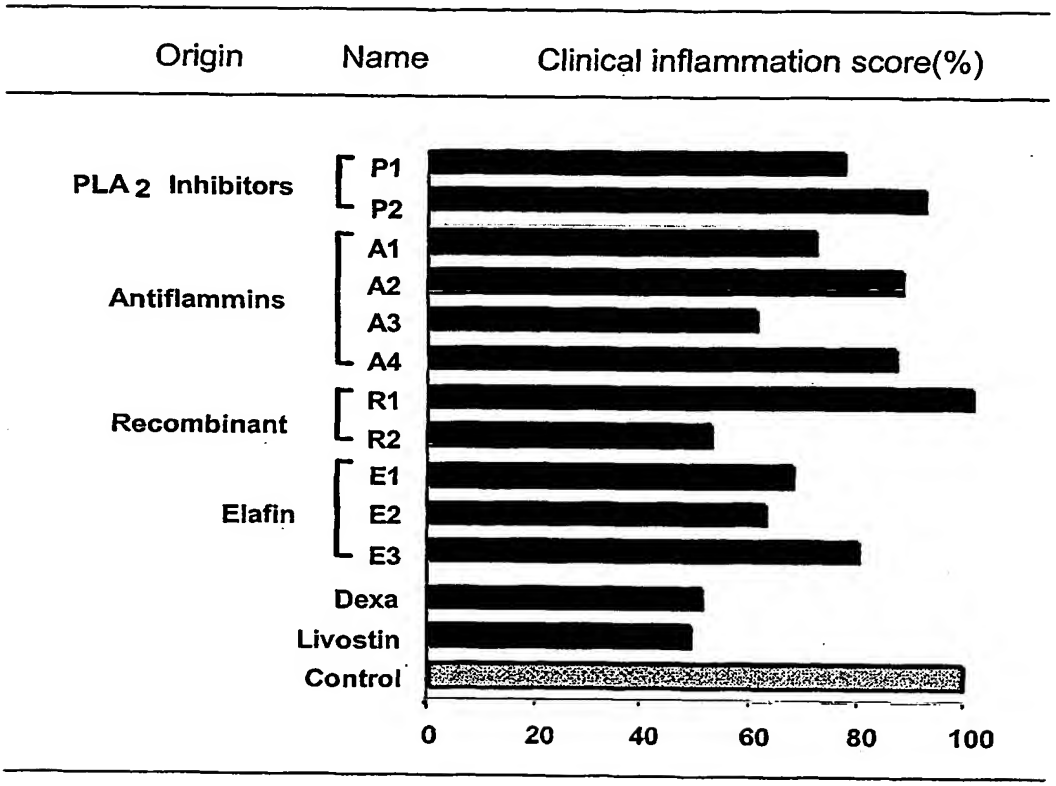
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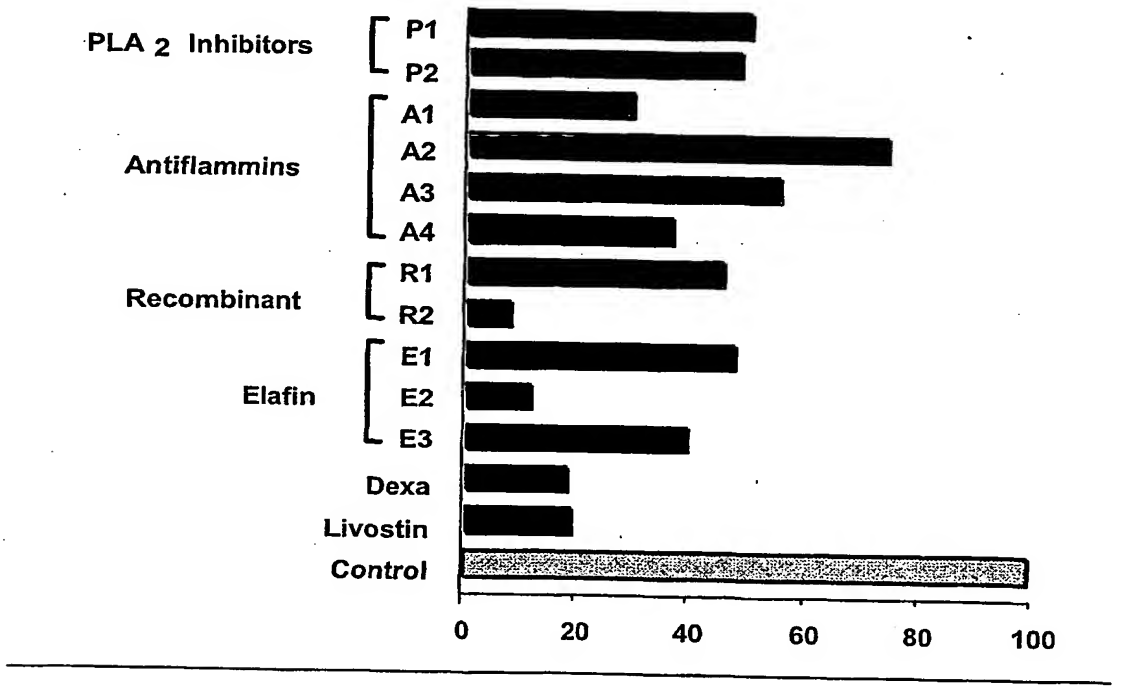
Fig. 5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E1
E2
E3
Dexa
Livostin
Control
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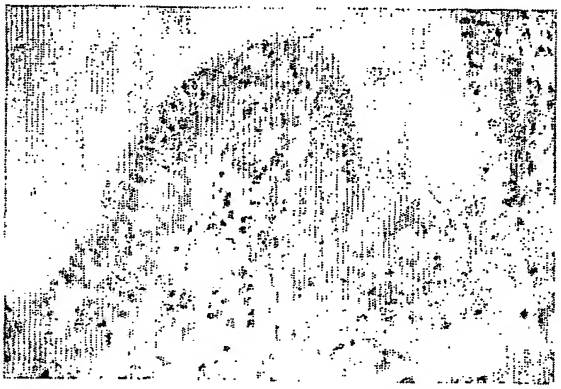
20
40
60
80
100

Fig. 6

H&E staining of conjunctiva; normal control (A), inflamed conjunctiva by pollen with no treatment (B), with R2 (C), with E2 (D), with dexamethasone eye drops (E) , and with Livostine (F)

(A)



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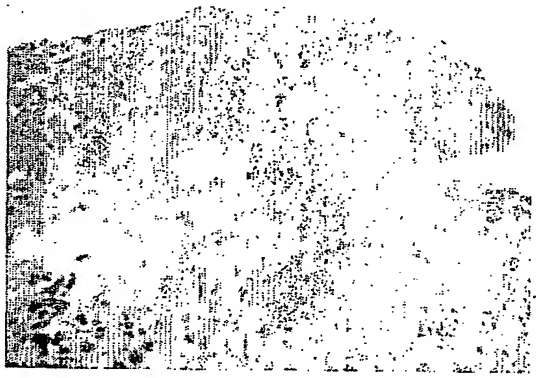

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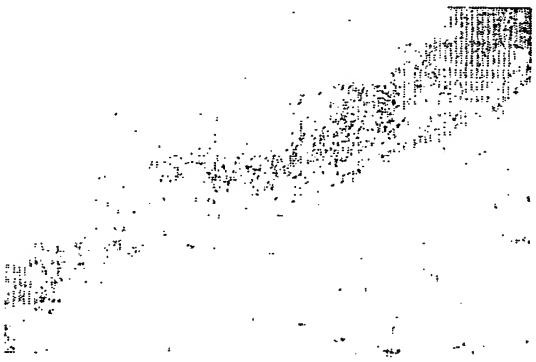

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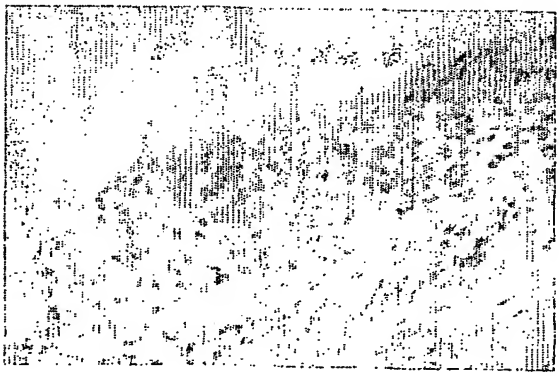

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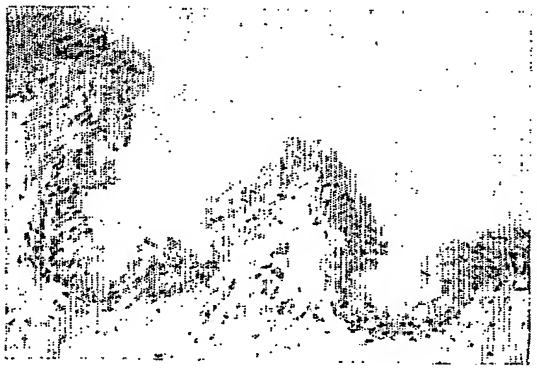

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00536

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 7/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NCBI GenBank, PubMed, CA, USPTO, Espacenet, PAJ, "peptide", "antiinflammatory activity", "transglutaminase", "phospholipase"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92/06997 A1 (Teijin Ltd.) 20 Apr. 1992.	1-9
A	JP 59029646 A2 (Fujisawa Pharm. Co. Ltd.) 16 Feb. 1984.	1-9
A	JP 06211684 A2 (Kao Corp.) 02 Aug. 1994.	1-9
A	JP 06263796 A2 (Teijin Ltd.) 20 Sep. 1992.	1-9
A	US 623690 A (National Institute of Health) 01 Aug. 1991.	1-9

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 JUNE 2002 (25.06.2002)	25 JUNE 2002 (25.06.2002)

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
LEE, Cheo Young
Telephone No. 82-42-481-5594

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/00536

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7-9
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 7-9 directed to a method of treatment of the human, the search has been carried out and based on the alleged effects of the peptides of claims 1-5.

2. [ ] Claims Nos.:
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. [ ] As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/00536

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 92/06997 A1	20 Apr. 1992	US 5,374,713 A	20 Dec. 1994
		EP 506971 A1	07 Oct. 1992
		AU 8716491 A1	20 May 1992